# Evaluation of a Methyl Anthranilate-Based Bird Repellent: Toxicity to Channel Catfish *Ictalurus punctatus* and Effect on Great Blue Heron *Ardea herodias* Feeding Behavior

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#### **Abstract**

We evaluated the methyl anthranilate-based bird repellent, ReJeX-iT TP-40<sup>®</sup> (TP-40), for 1) its toxicity to channel catfish fingerlings (catfish), Ictalurus punctatus; 2) its effect on great blue heron (heron), Ardea herodias feeding behavior; 3) its efficacy in reducing heron predation on catfish; and 4) its effects on catfish growth. TP-40 effectively maintained MA concentrations near the water's surface and below toxic levels for catfish in the water column. Water samples collected from 0.03 and 0.35 m below the surface of catfish rearing tanks (6,664 L) treated at application rates up to the equivalent of 200 kg/ha contained less than 5 ppm active ingredient, which is less than the no observable effect limit for catfish fry. No treatment-related mortality of catfish was observed. Handling times of catfish fingerlings captured by herons from tubs treated with TP-40 initially increased at application rates of 19.6 kg/ha or greater but decreased as a function of the number of catfish captured. Under simulated aquaculture conditions, TP-40 did not affect the number of catfish eaten by herons from ponds treated at surface application rates of 0, 2, 22, and 220 kg/ha. TP-40 had no affect on the time herons spent handling live or dead catfish. Ponds treated with TP-40 at 220 kg/ha had a 46% increase in visibility (secchi disk method) and a 58% reduction in total suspended solids, suggesting the formulation was phytotoxic. TP-40 did not affect fish growth. Analysis of individual behavior showed that herons may have habituated or become indifferent to the effects of the repellent after repeated exposure. Under the conditions of the study, herons did not maintain their body weight unless catfish were made available by disease or supplemental feeding, suggesting that herons may be inefficient at capturing healthy catfish. Surface applications of TP-40 at 20 to 220 kg/ha were not effective in limiting predation by herons.

Production of catfish *Ictalurus punctatus* has increased 121% over the past 10 yr, with round weight production in the United States totaling 214 million kg in 1996 (USDA 1997). Approximately 80% of the

producers' sales of \$368 million were concentrated in four southern states: Mississippi, Arkansas, Alabama and Louisiana. Among concerns of producers are losses attributable to birds. Approximately 70% of

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catfish producers surveyed indicated that bird predation on their aquaculture stocks was a serious problem (USDA 1998). The primary species reported as problems by producers are double-crested cormorants *Phalacrocorax aurtius* (53%) and great blue herons *Ardea herodias* (42%).

The potential for economic losses to catfish production has been documented for these species (Stickley et al. 1992, 1995; Glahn and Brugger 1995), but methods for limiting bird predation on fish are limited because of costs, impracticality, or lack of effectiveness (Mott and Boyd 1995). Chemical repellents, which have been successfully used against birds for protection of other agricultural commodities (Mason and Clark 1992), have not been used in an aquacultural setting.

ReJeX-iT® bird repellents are commercially formulated products containing the well-described bird repellent methyl anthranilate (MA). Methyl anthranilate was first characterized as having bird repellent properties by Kare (1961). Considerable evidence for MA's mode of action and efficacy as a bird repellent in a variety of agricultural and non-agricultural uses has been amassed during the intervening 37 yr (Mason et al. 1989; Mason and Clark 1992). Methyl anthranilate acts as a primary repellent (Rogers 1974). Primary repellents do not require learning, because they are based on congenital avoidance of irritating stimuli (Clark and Mason 1993). Successful delivery strategies target the animal's receptor fields, such as mucous membranes of the eyes, mouth and nose.

Although MA can be safely ingested by terrestrial animals (Furia and Bellanca 1975), it is toxic to some aquatic organisms (Clark et al. 1993). This is a potential problem at aquaculture facilities because the concentrations required for avian repellency would also be lethal to fish. TP-40 was developed to overcome this problem by binding MA into a "repellent film" that can be dispersed across the waters surface. The design of the formulation limits diffusion of

MA into the water column, concentrating MA on the water's surface where it is likely to maximize delivery of the repellent to birds.

We conducted laboratory studies to evaluate the acute toxicity of TP-40 to catfish and to evaluate bird responses as a function of concentration. We also evaluated TP-40 under simulated aquaculture conditions to determine whether TP-40 reduces great blue heron predation on catfish, and to determine its effect on catfish growth and mortality.

### Materials and Methods

Study Animals

We trapped 18 herons from July-September 1996, near Greenwood, Mississippi, USA, on commercial catfish ponds using methods described by King (in press). Birds were transported to the National Wildlife Research Center (NWRC), Mississippi Field Station where they were examined for injury and physical condition, weighed, aged (juvenile or adult), wing clipped, and marked with color- and number-coded patagial wing tags to allow for individual identification (Day et al. 1980). Herons were observed daily for visible signs of illness or injury, and given physical examinations every 2 wk.

Herons were quarantined for a minimum of 14 d in a 0.18-ha holding pen with a 0.04-ha pond. This period was designed to give the herons time to adjust to their new environment and to familiarize the herons with foraging in the ponds. The pond was designed to similar specifications as found in commercial aquaculture ponds. The pond had a 0.1-m diameter fill and drain structure to drain, refill, and control water level. Covered roosts with Drydek<sup>®</sup> floors and wooden perches provided shade and protection from inclement weather.

The holding pen pond was stocked with 3,000 (30,000/0.4 ha) fingerling catfish (9.9–21 cm) as forage during acclimation. Nonetheless, the herons did not maintain

their body weights through foraging for live catfish. Therefore, we supplemented the feeding regimen by providing quarantined herons a minimum daily maintenance ration of 8% of each bird's body weight per d of dead catfish placed in the pond (Bennett and Hart 1993). Consumption of the maintenance ration was assumed based on the lack of dead fish in the pond at the subsequent feeding or observation session. Additional catfish fingerlings were stocked in the holding pen pond every 2 wk to maintain a forage source. Despite these efforts five herons died during the acclimation period due to a combination of factors including handling stress, malnourishment, or aggressive interactions between herons. The remaining 13 herons were used as test subjects.

# Effects of TP-40 on Fish Mortality— Lab Study

To determine the toxicity of TP-40 we placed 50 fingerling catfish obtained from commercial suppliers into a 6,664-L holding tank at the Mississippi State University aquaculture unit, and held the fish for adaptation and observation for 4 d. The number of incidental mortalities were noted. The diameter of the tank was 3 m and served as the basis for calculating the application rate of TP-40. The depth of the water was maintained at 0.9 m by a surface drain. At 0800 h the aerator to the tank was turned off and the temperature and dissolved oxygen content of the water were noted. Water samples were taken at depths of 0.03 and 0.35 m from the surface to quantify the amount of MA in the tank at the two depths. Samples were preserved with 0.1-ppm sodium azide, an aerobic metabolic poison, that inhibited microbial degradation of MA (Aronov and Clark 1996). TP-40 was then applied to the surface at one of the following application rates: 0, 2.2, 20, 40, 100, or 200 kg/ha. The formulation could be seen as an oily sheen covering the water's surface. We observed catfish periodically over the next 12 h and noted changes in catfish behavior and any mortality. At 2000 h the temperature and DO content of the water were noted and water samples at 0.03 and 0.35 m were taken and preserved as described above. After water quality was checked, a continuous stream of well water was pumped into the tank and the aerator was turned on. This manipulation cleared the tank of the surface application of TP-40. For each of the next 5 d the process was repeated with the level of treatment increased. Thus, the sequence of testing ranged from the control condition (0 kg/ha) to a maximum application rate of 200 kg/ha.

In addition to the 50 free-ranging catfish, an additional three catfish were held near the surface of the tank in a flow-through PVC pipe. These fish served as sentinels for the toxicity of TP-40 near the surface, where concentrations of MA were expected to be highest.

# Effects of TP-40 on Heron Feeding Behavior—Pen Trials

Great blue herons were used as an avian model for the evaluation of the formulation because they frequently forage at catfish farms, particularly along pond edges where a repellent film is likely to concentrate due to wind and water motion. We observed feeding behavior of herons presented with two tubs containing catfish. Tubs (113-L rubber containers) were placed 10 m apart in the holding facility. In one tub, we placed 10 catfish fingerlings (0.07-0.15 m) and applied TP-40 at one of the following surface application rates: 0, 4.3, 8.6, 19.6, 38.3, 81.5 or 163 kg/ha. The second tub served as an untreated control. After treatment, the observer retreated from the holding facility and drove to a nearby hill approximately 150 m distant to observe feeding behavior using binoculars and a vehicle as a blind.

It took between 30–120 min for the herons to "discover" the tubs. Individual interactions among herons generally were restricted to acquiring a feeding perch on the rim of the tub. Once positioned other her-

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ons, while remaining at the periphery, did not overtly interact with the dominantly positioned heron. Thus, striking at catfish in the tub and handling the fish once caught was not interfered with by other birds. Only a single treatment level was tested each day (ca. 1700-1900 h). The order in which the surface application rate was tested across days was determined randomly. Because of the constrained nature of the experiment, once a heron established itself at a feeding perch it remained there until all fish were consumed, regardless of whether the tub contained TP-40 or not. Thus, we report only the total time it took to empty the tubs of fish and the handling time for individual catfish as a function of experimental conditions (i.e., treatment type and concentration). Handling time was defined as the time (s) it took an individual heron to kill, manipulate and swallow a fish starting from the time of a successful strike.

#### Field Test Protocol

After quarantine, four birds were introduced into the test facility based on physical condition and whether they had previously been used in a test. The test facility consisted of a 0.18-ha enclosure that contained two 0.04-ha ponds. The test ponds were divided by a mesh barrier with 4-mm thick plastic to 0.6 m below the pond surface. The barrier was designed to prevent movement of fish between pond halves, and to restrict upper water column circulation. Divided ponds provided four 0.02-ha test pond halves. Bubble-type aerators were placed near the center of each pond half and in the holding pen pond to maintain dissolved oxygen (DO) levels at 3 ppm or greater.

TP-40 was applied at four application rates, 0 kg/ha (control), 2 kg/ha, 22 kg/ha, and 220 kg/ha. These application rates encompassed the proposed labeling directions of the manufacturer (22 kg/ha). The application rates were assigned to the pond halves in a complete block design with each pond half receiving a different treatment level. Four 14-d replications were conduct-

ed with each pond half receiving all application rates over the course of the study. However, the effects of different application rates of TP-40 on catfish inventories, observed predation, and fish growth are reported for replications 1 and 2 only because of a severe die off due to enteric septicemia of catfish (ESC) in replication 3, and a probable fish stocking error in replication 4.

Previous studies indicated rapid degradation (<24 h) of the active ingredient in TP-40 (Aronov and Clark 1996; I. Mezine, Monell Chemical Senses Center, personal communication), indicating the need for intensive application to maintain treatment levels. In a preliminary test the MA concentration in water was assessed as a function of water depth, time, and surface application rate (2 kg/ha, 22 kg/ha, and 220 kg/ha) of TP-40 in replicated enclosures contained within catfish rearing ponds. Each sampling enclosure had a larger surface to volume ratio than for the catfish ponds. Accordingly, the concentrations of MA in the water column for the enclosures were expected to be higher and therefore, conservative with respect to that for test ponds.

We applied TP-40 to ponds for the 2 kg/ ha treatment with a 250 mm Nalgene squirtbottle. We applied TP-40 for the 22 and 220 kg/ha treatment levels with a hand-pump sprayer. We sprayed within 1 m of pond margins. We treated ponds every other day at 1500 h, starting the first day of the replication period. At the end of each replication, the ponds were flushed, drained completely, and refilled to remove any remaining MA. MA concentration and degradation rate were monitored at the surface and within 3 cm of the bottom of the ponds every 24 h for replication 4. Samples were taken at 1400 h every day, prior to application of TP-40. All chemical analyses for MA followed procedures developed by Clark et al. (1993) and Aronov and Clark (1996).

MA is phytotoxic and toxic to aquatic invertebrates (Askham 1992; Avery 1992). As a consequence of the toxicity of dissolved MA, the clarity of water may be in-

creased. To quantify the effect of MA on water clarity, we took secchi disk readings of the highest concentration pond and the lowest concentration pond at the termination of the study. In addition, a dry weight measure of total suspended solids (TSS) was taken by filtering a 150-mL sample of pond water from the two ponds.

### Stocking Rates and Treatment Effects on Catfish—Field Test

Each 0.02-ha pond was stocked with 1,500 fingerling catfish at the start of each replication (i.e., 30,000 fingerlings per 0.4 ha). Fingerlings of this size were used because they were considered the most vulnerable and preferred size class of catfish exploited by great blue herons (Stickley et al. 1995).

We monitored DO content of the water on a daily basis. Aerators were activated if DO fell below 3 ppm. Catfish were fed twice daily, at 0700 h and 1300 h EDST, at a daily rate equivalent to 3–5% of the average body weight, using a 32% protein, floating feed typically used in commercial aquaculture operations.

Because weight is often used as an index of the health of fish (Ney 1993; Devries and Frie 1996), we weighed random samples of catfish from each 0.02-ha pond at stocking and at the end of each test to evaluate possible effects of TP-40 on growth of fingerling catfish. Changes in weight over the 2-wk observation period were compared as a function of application rate using a fixed effects, one way analysis of variance (AN-OVA) (SAS Institute 1994).

We compared fish losses (number of fish stocked-number of fish remaining at the end of the test) among application rates. At the end of each replication, we drained the ponds and counted the number of catfish. To account for positional and temporal bias, losses were compared using a mixed model ANOVA, in which treatment level was the fixed effect and replication and pond were random effects (SAS Institute 1996). Any potential differences among treatments

might be attributable to direct toxic effects of TP-40 on fish, to differential predation rates on fish by herons, or to "natural" mortality sources (i.e., disease, parasitism, cannibalism). Inferences about the cause of potential fish losses were made daily by monitoring ponds for die-offs of catfish and by observing feeding behavior of herons.

### Effects of TP-40 on Behavior of Herons— Field Test

We assessed the effect of TP-40 on feeding behavior of herons. At the start of each replication, four herons were placed in the test enclosure. There were no physical barriers to prevent herons from choosing freely among the four treatment levels associated with the four different 0.02-ha pond halves. Differences for the behavioral measures among the application rates (treatments) were measured as a 2 factor mixed model ANOVA, with treatment level, fish status (alive or dead), and treatment level by fish status interaction term as fixed effects and pond and replication as random effects (SAS Institute 1996).

We observed herons with  $7\times50^{\circ}$  binoculars from a camouflaged platform approximately 50 m from the test facility for 1 h, twice daily (0800–0900 and 1100–1200, or 1600–1700 and 1900–2000 h, on alternating days) throughout each 2-wk trial. During each hourly observation period, individual patagially marked herons were randomly targeted for focal observation for 15 min.

Event behavioral measures recorded included the number of fish captured and swallowed, and whether the fish was alive or dead at the time of capture. Duration behavioral measures recorded included the handling time for processing fish. We defined handling time as the interval from capture of a fish to its being swallowed. Correlation between the numbers of fish observed eaten and the numbers of fish missing at inventory were analyzed. A correlation between the numbers of catfish observed eaten and counted at the end of each replication, by treatment level, would lend

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Table 1. Mortality of fingerling catfish during a 12h observation period as a function of surface application rate of TP-40.

Application rate (kg/ha)	Mortality out of $N = 55$	Rate (%)
0	3	5.5
2.2	0	0
20	1	1.8
40	0	0
100	0	0
200	0	0

validity to the interpretation that TP-40 influenced predation rates.

During each replication we visually monitored herons for signs of possible malnourishment. If malnourishment was evident, a program of supplemental feeding of half the recommended daily maintenance ration ( $\frac{1}{2} = 4\%$  of body weight) was provided to the birds (Bennett and Hart 1993). The half daily ration would be given at least 24 h prior to the next observation period to minimize any effect on foraging behavior.

#### Results

## Effects of TP-40 on Fish Mortality— Lab Test

There was no apparent relationship between short-term mortality of catfish and application of TP-40 to the water's surface for any of the application rates (Table 1). The MA concentration at the midpoint in the water column was less than 5 ppm for even the high application rate (Fig. 1). Periodic observations during the 12-h exposure periods indicated that catfish behaved normally and kept mostly to the bottom of the tank at a depth of 0.9 m; that is, the level of a shallow commercial impoundment.

There was no observed mortality for the sentinel fish (N = 3) maintained in a cage positioned within 7.5 cm of the surface although concentrations of MA were well within the LD50 concentrations of 20 ppm for catfish fry. Fig. 1 indicates that the MA content of water at the level of the sentinel

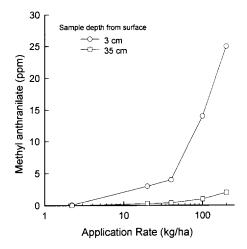


FIGURE 1. The MA concentration in a 6,664-L fish rearing tank 12 h after a surface application of TP-40 as a function of application rate and sample depth.

fish was about 15 ppm or greater for the 100 and 200 kg/ha application rates.

### Effects of TP-40 on Heron Feeding Behavior—Pen Trials

Great blue herons spent more time handling catfish extracted from tubs treated with TP-40 at rates of 19.6 kg/ha or higher relative to the controls (Fig. 2; analysis of variance F = 3.12, df = 1,5, P < 0.013). However, the average handling time for catfish extracted from treated tubs was not significantly different for surface application rates of 19.6 kg/ha or higher. Observations indicated that the handling time of fish extracted from treated tubs increased because of increased manipulation, wiping of fish on the ground, and head shaking. These observations suggest that the fish became coated with the surface film during the extraction process, and that herons attempted to process the fish prior to ingestion. Handling time decreased for application rates of 19.6 kg/ha or greater as a function of the number of times an individual heron struck at, and captured catfish (Fig. 3).

# Stability of TP-40 in Catfish Ponds— Field Test

Preliminary enclosure tests indicated concentrations of MA never exceeded 7

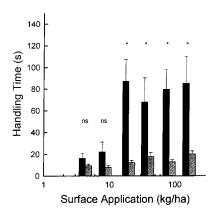


FIGURE 2. The mean handling time by great blue herons for catfish fingerlings as a function of TP-40 treatment concentration (solid bars) relative to simultaneously available untreated controls (shaded bars). The vertical capped bars depict one standard error of the mean. Asterisks depict post hoc comparisons (P < 0.05) between handling times for fish from the TP-40 treatment vs the handling time for the simultaneously presented untreated control tub. ns indicates a post hoc probability P > 0.05.

ppm, which is the no observable effects limit (NOEL) for catfish fry for any sampling depth for the application rates of 2 and 22 kg/ha (Fig. 4). The concentration of MA for the 220 kg/ha application rate exceeded the reported LC50 for catfish fry of 20 ppm for up to 3 d post-treatment throughout the water column (Fig. 4). The concentration of MA approached the NOEL throughout the water column by the fourth day after treatment (Fig. 4).

TP-40 applications on experimental ponds produced a visible iridescent film on the surface of the water, but even at high application rates this film appeared to be uneven. The surface film was primarily concentrated along the pond edges. Stability tests for TP-40 for the 0.02-ha pond halves indicated the MA concentrations in the water column reflected the magnitude of the application rate on the water's surface (compare panels in Fig. 5). The MA concentration throughout the water column remained below the NOEL for catfish fry for the 2 and 22 kg/ha treatments throughout replication 4, despite reapplication of TP-

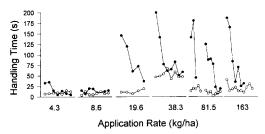


FIGURE 3. The handling time by great blue herons for live fingerling catfish as a function of order of capture by individual herons. At the lower application rates (4.3 and 8.6 kg/ha, solid circles) the order of capture had no effect on handling time relative to handling times for catfish from untreated tubs (open circles). At the higher application rates (≥19.6 kg/ha) handling time decreased as a function of the capture order, i.e., fish caught first took longer to process). Note that for the 81.5 kg/ha application rate, that two herons visited the tub sequentially. Each showed the ordering effect.

40 every other day. For the 220 kg/ha application rate, MA concentration remained below the NOEL for catfish fry at a depth of 0.6 m. Concentration of MA remained below the LC50 for catfish fry (20 ppm) for the 220 kg/ha treatment even near the sur-

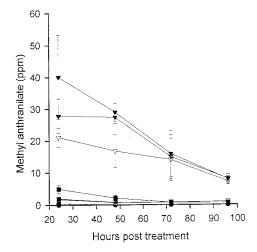


FIGURE 4. Stability of TP-40 formulation in field enclosures as a function of treatment level, time, and water depth. MA content in the water column is depicted at 0.03 m (solid symbols), 0.35 m (shaded symbols), and 0.6 m (open symbols) for three different surface application rates: 220 kg/ha (inverted triangles), 22 kg/ha (squares), and 2 kg/ha (circles). Vertical capped bars depict ±SEM.

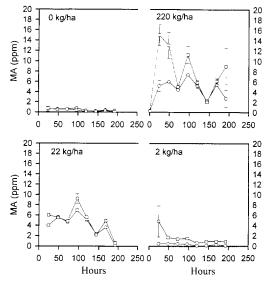


FIGURE 5. MA content of the water column in catfish ponds at depths of 0.03 m (squares) and 0.6 m (circles) as a function of surface application rate of TP-40 at 48-h intervals in replication 4. Water samples were taken every 24 h post treatment. Panels depict physical placement of treatments among the four 0.02-ha ponds. The vertical dotted lines depict the barrier between 0.02-ha ponds. Vertical capped bars depict ±SEM.

face. These data are consistent with our observations that no catfish mortality was associated with treatment of the ponds' surfaces. However, after repeated applications at the high application rates, water clarity

visibly increased. Secchi disk visibility readings for the high (220 kg/ha) and low (2 kg/ha) concentration ponds were 54 cm and 37 cm, respectively. The TSS for the high and low concentration ponds were 0.14 g/L and 0.22 g/L, respectively.

# TP-40 Effects on Catfish Growth and Mortality, and Heron Predation— Field Test

TP-40 did not appear to directly cause catfish mortality or affect growth rates. All catfish grew equally well during the 2-wk trial, irrespective of TP-40 application rate (F = 1.53, df = 3,4, P = 0.336). Average weight gain by catfish was 2.8, 1.3, 1.7, and 0.78 g for the 0, 2, 22, and 220 kg/ha application rates, respectively (Table 2).

TP-40 did not protect catfish from predation by herons. Although the numbers of catfish recovered from the ponds after each of the 2-wk trials were substantially reduced, we found no correlation between the numbers of catfish observed eaten from ponds treated at specific application rates and decreased catfish inventories in those respective ponds (P = 0.45, R = 0.27, N = 8). Nor did we find evidence that the catfish inventories differed among the treatment rates (F = 3.37, df = 3,1, P = 0.38). Overall, catfish numbers decreased by 1,089

Table 2. Pre-test and post-test mean catfish weights, sample size (N), standard error, and percent gain (+) or loss (-) in catfish weight for replication and treatment level.

	Pre-test				Post-test		
Concen- tration (kg/ha)	Mean Weight N (g)		Standard error	N	Mean weight (g)	Standard error	Weight gain or loss (%)
			Rep	olication 1			
0	50	8.5	0.1	50	12.5	0.1	+47.0
2	50	8.8	0.1	50	10.3	0.1	+17.0
22	50	7.9	0.1	49	9.1	0.1	+15.1
220	50	7.7	0.1	50	8.5	0.1	+10.4
			Rep	olication 2			
0	50	10.4	0.1	50	11.9	0.1	+14.6
2	50	10.4	0.1	50	11.4	0.1	+9.6
22	50	9.7	0.1	50	11.8	0.1	+22.0
220	54	10.6	0.1	50	11.4	0.1	+7.5

TABLE 3. Numbers of fish stocked, fish counts at the end of each replication, and total change in number.

Treatment level (kg/ha)	Number stocked	Number recovered	Net change
	Replic	cation 1	_
0	1,500	1,181	319
2	1,500	1,079	421
22	1,500	1,342	158
220	1,500	1,309	191
	Replic	cation 2	
0	1,500	1,293	207
2	1,500	1,415	85
22	1,500	1,185	315
220	1,500	1,059	441

(-18.2%) and 1,048 (-17.5%) from the initial number of 6,000 during replications one and two, respectively (Table 3). The average decrease in the number of fish per application rate was 202 (±117 SEM), 314 (±107), 299 (±141), and 253 (±62) for the 0, 2, 22, and 220 kg/ha, respectively (Table 3). Because catfish losses from controls were similar to those from ponds treated with TP-40, we conclude that TP-40 had no discernable impact on fish losses, and that such losses were probably attributable to indiscriminate foraging by herons between ponds and natural mortality sources.

TP-40 had no detectable effect on pre-

dation success or handling time by herons. The number of catfish directly observed by us to be eaten by herons did not differ as a function of application rate (F = 2.18, df 3,1, P = 0.45). Nor did application rate differentially affect the time required by herons to process live or dead catfish (F = 0.34, df = 3,28, P = 0.79). However, herons took longer to handle and process live catfish than dead catfish (Fig. 6, F = 5.68, df = 1,28, P = 0.02).

Although herons were observed to forage on ponds, they did not maintain their body weights over the 2-wk test. A physical examination of herons indicated poor condition necessitating the replacement of one bird midway through the first replication. The average weight loss for the three birds that remained on test for the full 14-d period was 22%. Herons in replication 2 appeared to be having little success in maintaining their body weight and were supplementally fed half the daily maintenance requirement (4% of their body weight) over the last 6 d of replication 2. With supplemental feeding, herons in replication 2 lost an average of 4.8% of their body weight. In replications 3 and 4 the herons appeared to maintain adequate condition and no supplemental feeding was needed. Mean weight loss for herons was 10.9% and 7.3% for tests 3 and 4, respectively.

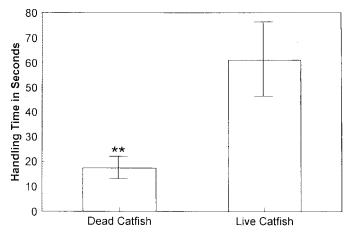


FIGURE 6. Mean handling time of live and dead catfish. Vertical capped bars depict  $\pm$ SEM. Asterisks represent statistical significance at the P=0.05 level.

#### Discussion

The MA concentration at the midpoint in the water column of catfish rearing tanks was less than the NOEL for catfish fry, i.e., 7 ppm (Clark et al. 1993), for even the highest application rate and presumably was less at the bottom of the tank where the fish spent most of their time. Thus, it is not surprising that a concentration-dependent mortality was not observed in the catfish rearing tanks. More surprising was the absence of mortality for the sentinel fish maintained in a cage positioned within 7.5 cm of the surface. The concentrations of MA were well within the LD50 concentrations reported for catfish fry, i.e., 20 ppm (Clark et al. 1993). Perhaps the acute insensitivity of these sentinel fish to the lethal effects of MA is attributable to their larger biomass relative to fry. This point remains to be determined. The partition coefficient for MA between octanol and water is 84 (Aronov and Clark 1996), where as the partition coefficient for MA between TP-40 and water is 178 (L. Clark, USDA National Wildlife Research Center, and E. Aronov, Monell Chemical Senses Center, personal communication). That is to say, the formulation is designed to have a high affinity for MA, such that diffusion of MA into the water column is curtailed. Our lab test data suggest that TP-40 acts according to the objectives of the formulation.

Performance characteristics of TP-40 in the field tests relating to fish toxicity were concordant with those of our pilot laboratory studies. TP-40 maintained MA concentrations near the water's surface and below toxic level for catfish in the water column following the anticipated design specification of the manufacturer. TP-40 had no significant effect on short-term growth of catfish. In addition, there were no obvious behavioral or physical signs of illness in catfish, and catfish mortality was not observed to increase in association with application of TP-40 at any level.

During pen trials, handling time decreased as a function of the number of times individual herons struck at and cap-

tured catfish for application rates of 19.6 kg/ha or greater (Fig. 3). There are two possible explanations for this behavior. First, the wicking action of feathers may have extracted the surface application of the formulation such that repeated, sequential capture attempts eventually depleted the amount of repellent on the water's surface. Thus, each subsequently extracted fish was coated with less formulation. This scenario is unlikely because the handling time of each fish was independent of application rate, suggesting that fish were equally coated by the repellent formulation. A second possibility is that herons became desensitized to the repellent with rapid, repeated exposure. Fig. 3 shows that for the 81.5 kg/ ha application, two different herons foraged from the same tub; the first heron was displaced by a second heron. The first heron showed the typical pattern for decrease in handling time as a function of capture sequence. The second heron showed the same pattern. If the formulation had been depleted as a function of number of strikes, then the pattern for the second heron should have been diminished relative to that of the first heron's. The parallel patterns for handling times for the two herons is more suggestive of desensitization and/or increased tolerance to the effects of the irritant qualities of the repellent.

These data suggest that TP-40 might have the advertised effect on heron behavior. Even though all fish were consumed, we did not regard this outcome as necessarily damaging to the desired performance of the repellent. Tub studies and closely-confined pen studies often present animals with a no or forced choice situation that tend to diminish the putative effects of repellents (Mason and Clark 1995, 1996; Belant et al. 1996). The aversive qualities of repellents are more effective if the animal has a choice to remove itself from the situation or exploit alternative resources.

One striking feature of the results of field tests, and one unanticipated in the study design, was that great blue herons did not maintain their body weights under test conditions that imitated standard aquacultural practices. The high densities of herons and the mix of adult and juvenile birds may have resulted in unusually high levels of agonistic behavior that reduced foraging time or success of subdominant herons. All herons lost weight during all replications. However, weight loss was greatly reduced when herons were fed supplementally half of their daily maintenance ration or when an event such as a disease outbreak made dead or dying catfish available. In addition, herons held in the holding pens were maintained in good condition when fed a maintenance ration of channel catfish. These data suggest that herons may not forage efficiently on catfish unless some event such as disease, low dissolved oxygen, or feeding of floating feeds makes catfish available. This interpretation is consistent with field studies of heron predation that indicate that herons foraging at catfish ponds supplement their diet with dead catfish and species other than catfish (Stickley et al. 1995). In addition, reduced handling times for dead catfish versus live catfish suggest benefits in terms of net energetics for herons targeting dead catfish. Considering the possibility of poor foraging efficiency of herons on healthy catfish, further studies are needed to clarify the impact of herons on catfish production.

Although the active ingredient (MA) in TP-40 is irritating to avian species (Kare 1961; Mason et al. 1989), herons were not affected by treatment with TP-40 at any level under simulated aquaculture conditions. Repeated application rates an order of magnitude greater than the recommended level did not deter herons from consuming catfish from ponds. In addition, handling time for herons consuming catfish was not significantly affected by treatment with TP-40, suggesting no interference with normal foraging activity on ponds. These observations are in contrast to expectations based on our more restricted pen observations for the effects of TP-40 on feeding behavior of herons.

Distribution of TP-40 on the surface of ponds was uneven. Because herons stand or wade along the shoreline and strike at a relatively small area they may not always be exposed to the repellent. Additionally, the formulation's ineffectiveness may relate to its phytotoxicity. Cezilly (1992) found increased turbidity significantly reduced prey capture rates for little egrets *Egretta garzetta*. Conversely, prey capture may be increased by increased water clarity. However, the phytotoxic and zootoxic effects of TP-40 on heron foraging efficiency in this study were not apparent and would require further research.

These data provide valuable information about the utility of TP-40 for use in alternative delivery tactics, e.g., repellent fogs or aerosols. If such delivery tactics are to be further developed, some assurance is needed that TP-40 is not harmful to aquatic organisms. While this question needs further study, findings provided for by this study suggest that extraordinarily high levels of MA confined by the TP-40 formulation may provide at least some protection against the toxic effects of MA to aquatic organisms. However, while TP-40 did not harm catfish, it also did no good when applied as a surface-film bird repellent. Although the active ingredient has been shown to be irritating to avian species, the present formulation did not limit predation on catfish by great blue herons.

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